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THE RAT-BITE FEVER SPIROCHETE, WITH COMPARATIVE STUDY OF HUMAN, WILD RAT AND FIELD VOLE STRAINS

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In 1915 Futaki, Takaki, Taniguchi and Osumi[1] discovered in a swollen lymph gland of a rat-bite fever patient a spirochete which they regarded as the cause of this disease. A month later, Ishiwara, Ohtawara and Tamura[2] succeeded in finding the same spirochete in a guinea-pig experimentally infected with rat-bite fever, transmitted by the bite of a wild rat. Subsequently Kitagawa, Kato, Abe and Mukoyama,[3] Futaki and his associates,[4] Kaneko and Okuda,[5] and Row,[6] in India, each group working separately, again demonstrated the existence of this spirochete, making use of specimens obtained from human beings; and Ido, Ito, Wani and Okuda[7] showed that there is a specific relation between Futaki's spirochete and antibody in the blood of patients. Spirochaeta morsus-muris, as it was designated by Futaki and his associates,[8] has since been proved beyond doubt to be the causative agent of the rat-bite disease occurring in Japan.

In addition to the investigations just noted, there have been published in Japan several papers on the rat-bite fever spirochete. A number of contradictory statements in these publications have led us to repeat certain experiments; moreover our interest in the morphologic similarity between this spirochete and that found in the blood of the field vole by Miyajima has made a comparative study of these strains advisable.

For this experiment we selected four strains of the spirochete, the human 1 and 2,* the wild rat and the field vole strains.

Received for publication Dec. 9, 1918.

* We are indebted to Dr. Ido for the human strain 1 and to Dr. Kitagawa for the human strain 2.

[1] Tokyo Igakukwai-Zasshi, 1915, 29; Jour. Exper. Med., 1916, 33, p. 249.

[2] Tokyo Igakukwai-Zasshi, 1916, 30; Jour. Exper. Med., 1917, 25, p. 45.

[3] Igaku-Chuo-Zasshi, 1916, no. 126.

[4] Futaki, K., Takaki, I., Taniguchi, T. and Osumi, S.: Tokyo Iji-Shinshi, 1916, no. 1976; Jour. Exper. Med., 1916, 33, p. 249.

[5] Iji-Shimbun, 1916, nos. 951 and 952; Jour. Exper. Med., 1917, 26, p. 363.

[6] Ind. Jour. Med. Research, 1917, 5, p. 386.

[7] Jour. Exper. Med., 1917, 26, p. 377.

[8] Futaki, K., Takaki, I., Taniguchi, T. and Osumi, S.: Jour. Exper. Med., 1917, 25, p. 33.

DISTRIBUTION IN THE ANIMAL BODY

Seventeen guinea-pigs, 6 wild rats and 13 white rats were each inoculated with one of the four strains and killed between 8 days and 4 months after inoculation. All the organs of the animals thus treated were then examined, the preparations being stained by Levaditi's old method.

Our investigation showed the distribution of the spirochete to be the same for each strain in each animal. In the early stages of the infection the spirochetes are found principally in the blood, but after 2 weeks a large number appear in the connective tissue which as time goes on is gradually increased. The following is the arrangement of the organs in order according to the number of organisms found; the subcutaneous and submucous connective tissue and muscle of the eyelids, lips, bridge of the nose and tongue, the intermuscular connective tissue of the heart wall, the capsules of the salivary and lymph glands, the adventitia of aorta and large arteries of the testicles, lungs, kidneys, etc., the spleen, the liver, the adrenal glands, the kidneys, the parenchyma of the salivary and lymph glands, etc.

As far as we know, there has been no previous record of the constant occurrence of large numbers of spirochetes in the adventitia of the aorta and large arteries within the visceral organs, nor of their occasional abundant occurrence in the endocardium of the heart and their concentration in the reticular connective tissue of the vascular sinus surrounding the follicle of the tactile hair of the upper eyelids and lips.

Notwithstanding many workers have stated that they have found this spirochete in the excretory ducts and the secretory tubules of the salivary glands, and in the tubules of the kidneys, we have been unable to find the organism in these tissues.

ROUTE OF EXCRETION

As the name indicates, rat-bite fever is caused by the bite of a rat, but it is important to ascertain whether or not the spirochete in question can be discharged in the saliva. Accordingly the saliva of the infected guinea-pigs, wild rats and white rats was examined directly by microscopic examination of preparations of saliva, and indirectly by inoculation of saliva solution obtained by washing the mouth cavity carefully with about 0.3 c c of salt solution. In no case could we detect the existence of spirochetes in saliva by the first method, but the inoculation of mice with the saliva of 7 infected animals, 2 guinea-pigs, 3 wild rats and 2 white rats, yielded positive results only in the case of the mice inoculated with the saliva of one of the wild rats. We do not consider this one positive result sufficient evidence that the disease is caused by the excretion of the spirochete in the saliva; or through the normal mucous membrane of the mouth cavity, as is held by Futaki and his associates.

The frequent excretion of this organism in the urine has also been reported by many investigators. But, in view of our failure to find the spirochete in the tubules of the kidneys, it seemed necessary to repeat the investigations with regard to the urine. Nineteen infected guinea-pigs were killed and the urine taken aseptically from their bladders, a syringe with a burning needle being used. We then inoculated from 1-10 mice for each guinea-pig using about 0.25 c c of urine per mouse. From those 19 cases, killed at various stages after inoculation, we obtained only two positive results. The excretion of this organism in the urine, therefore, seems not to occur as frequently as is generally believed and to be indeed rather rare.

The next hypothesis to be tested was that of the excretion of the spirochete in the bile. We examined the bile of 10 infected guinea-pigs by the same methods as those used in the case of the urine, but with no positive result in any case, as was also the case in the experiments of Futaki and his associates.

We were similarly unable to find microscopically the spirochete in the intestinal contents of a number of guinea-pigs, wild rats, and white rats. The intestinal contents of an infected guinea-pig and a wild rat were inoculated into 4 guinea-pigs, but without positive results.

MODE OF INFECTION

The frequency of the occurrence of this infection in wild rats is 21 to 186 (11.2%) according to Ishiwara and his associates, 5 to 84 (5.9%) according to Matsuzaki and his associates,[9] 8 to 58 (13.8%) according to Tsuneoka[10] and we detected it in 2 of 24 wild rats. These results show that this form of spirochetosis is prevalent among the wild rats in Japan. Moreover, there is now no doubt that the disease is transmitted by the bite of the spirochete-bearing wild rat. This was conclusively proved first by the experiments of Ishiwara and his associates and later by others. Our experiments showed 5 positive cases among 7 guinea-pigs bitten by an infected wild rat.

The question therefore arises as to how in such cases this spirochete is transmitted from its source in the body of the host if not through the saliva. The infected wild rat, and also the infected guinea-pig, usually becomes very irritable and furiously bites any objects coming in its way, very often suffering an abrasion and even bleeding in the lips or gums. The spirochete is then given an opportunity of escape from the submucous tissue, or the circulating blood, through the defective point in the mouth. It is possible, therefore, that the spirochete may be thus transferred by the bite of an infected rat to the body of a healthy rat or even a human being. Nevertheless, it was desirable to ascertain whether this spirochetosis can be produced experimentally by other modes of transmission, as so many workers have supposed.

1. By keeping the infected and normal animals together in the same cages, we did not obtain a single positive result among 11 guinea-pigs, which were divided into 6 groups, each group being put with from 2-5 infected guinea-pigs. The same experiment was tried with 29 normal mice, which were placed in 7 cages, each with from 5-15 infected mice. Only 2 positive cases were observed, each in a different cage. In these experiments, the observation lasted from 1 week to about 1 month after inoculation in the case of the mice and 2 or 3 months in the case of the guinea-pigs. From the foregoing results, it is natural to conclude that these 2 positive cases, found only among the mice, might have resulted from the bite of the infected mice, and it seems far from possible, in spite of the assertion to the contrary of Haga and his associates,[11] that the spirochete can be easily transmitted, especially in the case of guinea-pigs, through the normal skin, or by ectoparasites, such as lice, fleas and mites.

2. By feeding clot densily contaminated by spirochetes to 24 mice, we obtained 4 positive results. In most cases these mice were kept in separate cages, in order to prevent possible infection by biting; but the 4 positive

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[9] Matsuzaki, H., Yamamoto, M., and Imai, K.: Tokyo Iji-Shinshi, 1916, nos. 1980 and 1981.

[10] Kyoto Igakukwai-Zasshi, 1917, 14, No. 4.

[11] Haga, I., Yoshizawa, K., Umemoto, E. and Hiroshige, J.: Nihon Eiseigakukwai-Zasshi, 1917, 13, no. 1.

results obtained occurred only among the mice which were put together and there were no positive cases among the mice which were separated. We found 2 positive cases among 14 similarly fed guinea-pigs.

3. By the instillation of blood containing large numbers of the spirochetes into the eye, we obtained only one positive case among 19 mice, which were kept in separate cages, and 4 among 14 instilled guinea-pigs.

From the results obtained in the preceding experiments in feeding and instillation of the organism, it seems hardly possible that it enters the animal body through the normal mucous membrane; for it is more than likely that the positive cases resulted from small abrasions. Therefore ,contrary to the opinion of Haga and his associates, we are certain that, even in the case of guinea-pigs, this infection does not readily occur through the digestive tract.

We believe that the natural and most important source of infection among wild rats is the bite of an infected rat, having a wound in the vicinity of the mouth. Infections by other means, under natural conditions, occur very rarely, if at all.

SYMPTOMS IN THE INFECTED ANIMALS

As many workers have already proved, the spirochete easily infects such experimental animals as the mouse, white rat, wild rat, guinea-pig and monkey; but one perceives a constant clinical change only in the guinea-pig and monkey, the other animals continuing apparently healthy, in spite of the abundant increase of the organism in their blood.

With regard to the clinical changes in the guinea-pigs and monkeys, infected with the human, wild rat, or field vole strain, we observed that in the guinea-pig, whether the inoculated strain be the human, wild rat, or field vole, the most important and constant symptoms are decrease in weight and alopecia. About 1 month after inoculation, the alopecia begins to appear around the face and at last spread over the whole body. The fever also is a constant symptom in guinea-pigs inoculated with the human or wild rat strain, but it seldom occurs in the case of the field vole strain.

In all cases of monkeys, especially Japanese monkeys, which we used as the experimental animals, and which we inoculated with the human or wild rat strain, we observed fever after about 1 week of inoculation, but, after several successive paroxysms, it usually subsided. In these cases we always detect the spirochete in the blood, if not microscopically, by inoculation. On the contrary, there was no reaction either microscopically or clinically in any of the 3 monkeys inoculated with the field vole strain. In this case, we were unable to credit the monkey's susceptibility to the field vole strain until it was conclusively proved by the immunologic reaction.

IMMUNITY RELATIONS BETWEEN THE HUMAN, WILD RAT AND FIELD VOLE STRAINS

The fact that specific antibody appears in the blood of rat-bite fever patients and has a spirocheticidal or lytic action on the causative spirochete has been demonstrated by Ido and his associates and by others. We also have tried to produce immunity in the animals experimentally and have succeeded in immunizing the Japanese monkey alone, no result being obtained in the case of the guinea-pig, white rat and mouse. This finding is consistent with the fact that the number of spirochetes in the body of the guinea-pig, white rate and mouse does not decrease, even after several months of infection, but rather increases in the connective tissues. On the other hand, we were unable to find the spirochete in the bodies of Japanese monkeys killed after recovery.

Being unable to obtain the serum of a patient, we used for the present investigation the serums of infected Japanese monkeys: monkey 18 inoculated with the human strain 2, monkey 19 inoculated with the wild rat strain, monkeys 1, 15 and 10 inoculated with the field vole strain and monkey 47 inoculated with the wild rat strain killed by exposure for 30 minutes to a temperature of 56 C.

Examination by Dark-Field Illumination.—The serum to be tested is first drawn into a capillary pipet up to a given mark. The blood taken from the tail of a heavily infected mouse is measured in the same way and the equal quantities of blood and serum thus obtained are thoroughly mixed by repeatedly drawing them in and out of the pipet. The mixture is then transferred to a slide, and examined under the dark-field microscope. The actions of the immune serums on the human and wild rat strains are shown in the following table.

TABLE 1

ACTIONS OF THE IMMUNE SERUMS ON HUMAN AND WILD RAT STRAINS

Serum	5 Minutes	15 Minutes	30 Minutes	1 Hour	Result
Human strain 2 serum (monkey 18)......	Motility languid	Rest	Rest	Rest	Positive
Wild rat strain serum (monkey 19)......	Motility languid	Rest	Rest	Rest	Positive
Field vole strain serum (monkey 1)......	Motility active	Active	A little languid	Rest	Weak positive
Field vole strain serum (monkey 10).....	Motility active	Active	Active	Active	Negative
Field vole strain serum (monkey 15).....	Motility active	Active	Active	Active	Negative
Japanese monkey 47 serum..............	Motility active	Active	Active	Active	Negative
Normal Japanese monkey serum 1........	Motility active	Active	Active	Active	Negative
Normal Japanese monkey serum 2.......	Motility active	Active	Active	Active	Negative

TABLE 2

ACTION ON THE FIELD VOLE STRAIN

Serum	5 Minutes	15 Minutes	30 Minutes	1 Hour	Result
Human strain 2 serum (monkey 18)......	Motility languid	Rest	Rest	Rest	Positive
Wild rat strain serum (monkey 19)......	Motility languid	Rest	Rest	Rest	Positive
Field vole strain serum (monkey 1)......	Motility active	Rest	Rest	Rest	Positive
Field vole strain serum (monkey 10).....	Motility active	Languid	Partly rest	Rest	Weak positive
Field vole strain serum (monkey 15).....	Motility active	Active	Active	Active	Negative
Japanese monkey 47 serum..............	Motility active	Active	Active	Active	Negative
Normal Japanese monkey serum 1.......	Motility active	Active	Active	Active	Negative
Normal Japanese monkey serum 2.......	Motility active	Active	Active	Active	Negative

The immune serums for the field vole strain have no action on the human and wild rat strains, as the control serums, except a slight action of the serum from monkey 1, while the immune serums for the human and wild rat strains have a distinct spirocheticidal action on their homologous strains.

The actions of these immune serums on the field vole strain are recorded in table 2.

Although the human strain and wild rat strain serums have a strong spirocheticidal action on the field vole strain, the field vole strain serums do not react in the same way to their homologous strain, except in the case of the serum from monkey 1.

TABLE 3

RESULTS OF EXPERIMENTS TO DETERMINE SPIROCHETICIDAL ACTION

Serum	Strain	Result
Human strain 2 serum (monkey 18)	Field vole strain Wild rat strain Human strain	Positive Positive Positive
Wild rat strain serum (monkey 19)	Field vole strain Wild rat strain Human strain	Positive Positive Positive
Field vole strain serum (monkey 1)	Field vole strain Wild rat strain Human strain	Positive Negative Negative
Normal Japanese monkey serum	Field vole strain Wild rat strain Human strain	Negative Negative Negative

TABLE 4

RESULTS OF EXPERIMENTS TO DETERMINE WHETHER OR NOT REINFECTION OCCURS

Monkey Number	Strain Injected at the First Inoculation	Strain Injected at the Second Inoculation	Interval Between First and Second Inoculation	Result
Monkey 18	Human strain 2	Human strain 2	68 days	Negative
Monkey 1	Field vole strain	Human strain 2	25 days	Negative
Monkey 10	Field vole strain	Human strain 1	80 days	Negative
		Human strain 2 (at the third)	132 days	Negative
		Human strain 2 (at the fourth)	150 days	Negative

Japanese monkey 47, inoculated with the killed rat strain, was infected typically by reinoculating the human strain.

To conclude, as far as examination of the slide is concerned, the field vole strain serum has no spirocheticidal action or only a slight one on the human and wild rat strains, and even on its homologous strain, while the human strain and wild rat strain serums always exert a strong spirocheticidal action on all these three strains.

Pfeiffer's Reaction.—Equal quantities of immune serum and infected blood are mixed and sodium citrate is added in a ratio of 1%. After being kept one hour at 37 C., the mixture is injected intraperitoneally into guinea-pigs. The result of this test is determined by the symptoms in the animal thus treated, by microscopic examination of its blood, and also, if necessary, by inoculation of the heart blood into mice.

The wild rat strain and human strain serums have a spirocheticidal action on all three strains, but the serum of the field vole strain has no positive action except on the field vole strain.

Examination by dark-field illumination and Pfeiffer's reaction, therefore, indicate the same fact, namely, that the field vole strain is apparently different from the other two strains, while the human and wild rat strains are evidently the same.

Reinfection Test in the Japanese Monkey.—We endeavored to ascertain whether or not reinfection occurs in the previously infected Japanese monkey on reinoculation of the human strain within a certain interval after the first inoculation.

The Japanese monkey, therefore, is not only made immune by the infection of the human strain, but it also develops immunity by the infection of the field vole strain, even enough to resist subsequent inoculation with the human strain.

We may conclude, therefore, from the foregoing immunological result, that the field vole strain is similar to both the human and the wild rat strains. The distinct difference in the virulence and immunizing power between the field vole strain and the others is probably due to the fact that, in passing through many generations of field voles, the spirochete decreases its toxophore and haptophore groups quantitatively.

Identity of Spirochaeta morsus-muris Futaki and Spirillum minor Carter, Spirochaeta laverani Breinl et Kinghorn, Spirochaeta muris Wenyon, etc.

Futaki and his associates regarded Spirochaeta morsus-muris as a new species, insisting that the presence of flagella and pathogenity in this spirochete differentiates it from the allied spirochetes referred to by other workers.

From table 5 as well as from the original figures of the workers, it is difficult to detect any difference in morphology, movement and pathogenity between Futaki's and the allied spirochetes, except for a slight difference of Spirillum minor.

As mentioned, Carter, Breinl and Kinghorn, and Wenyon could not prove the presence of flagella in their spirochetes, but it must be remembered that the process of dark-field illumination had not come into use in their time. This fact is especially noteworthy in the light of the subsequent discoveries of MacNeal, Mezincescu and Deetjen, who found flagella in their spirochetes by means of dark-field illumination.

Regarding pathogenity, there is also a slight distinction between Futaki's and the allied spirochetes. We must, however, again take into account the insufficiency of the animal experiments made by these workers and the variation in the virulence of spirochetes, which might be due very often to the difference in the species of their hosts, as has just been indicated in the case of the field vole strain.

It would seem therefore that Spirochaeta morsus-muris Futaki is, in all probability, similar to Spirillum minor Carter, Spirochaeta laverani Breinl et Kinghorn, Spirochaeta muris Wenyon, etc.

SUMMARY

In the infected wild rat, white rat and guinea-pig, the spirochete of rat-bite fever, in the early stages of the infection, are detected principally in the blood, but after 2 weeks a large number appears in the connective tissues, and as time goes on this number is gradually

TABLE 5

MORPHOLOGY AND PATHOGENESIS OF RAT-BITE FEVER SPIROCHETES

Name of Spirochete	Habitat	Length, μ	No. of Turns	Flagellum	Movement	Pathogenity
Spirillum minor[12] Carter (1887)	Indian rat (Mus decumanus)	5-9	4-8	No flagellum (in aniline-stained preparation)	"Very active and tolerably sustained, consisting of rotation round the long axis, propulsion either forward or backward, and occasionally an energetic lateral twisting or lashing."	Not transmissible to the rat and monkey (macacus)
Spirochaeta laverani[13] Breinl et Kinghorn (1906)	White mouse and wild mouse (Mus musculus)	1.8-3.75	2-4	No description of flagellum	"Actively motile, and shows both a movement of the spiral turns and a progressive movement of the whole organism."	Transmissible to the mouse and rat[14]
Spirochaeta muris[15] Wenyon (1906)	Brown mouse	3-7	2-6	"All its movements seem to indicate the presence of flagella though these were never actually seen."	"Moving along very rapidly, and turning on its long axis, it will suddenly come to rest and as suddenly dart off in an opposite direction."	Transmissible to the mouse and young rat, but not to the guinea-pig nor the adult rat
Sp. muris, var. Virginiana[16] MacNeal (1907) Sp. muris, var. Galatziana[17] Mezincescu (1909)	Wild rat (Mus decumanus)	1.75-3.55	1¾-3½	One at each end	"It appears as an elastic spiral rapidly darting about, as often with one end forward as the other, forth and back, rotating on its long axis as it goes. . . . At times a lashing movement of the whole body is seen."	Transmissible to the wild rat, white rat and house mouse (Mus musculus)
Spirochete found in the[18] mouse cancer by Deetjen (1908)	Mouse	1.5-5	1-5	One at each end	"Actively motile, and shoots to and fro, turns on both the long and transverse axes and turns over."	Transmissible to the mouse
Spirochaeta morsusmuris Futaki, Takaki, Taniguchi et Osumi (1915)	Human being	1.5-6	1.5-6	One at each end	"The movements are spasmodic, usually spiral, and freely pass backwards and forwards."	Transmissible to the human being, monkey, guinea-pig, wild rat, white rat and mouse, especially pathogenic for the first 3

increased. That is, this spirochete is always distributed numerously in the subcutaneous and submucous tissues of the eyelids, lips, bridge of the nose, and tongue, and is especially abundant in the reticular connective tissue of the vascular sinus surrounding the follicle of the tactile hair of the upper eyelids and lips. It is also usually, if not always, found abundantly in the capsules of the salivary and lymph glands, in the heart wall, in the adventitia of the aorta and large arteries within the visceral organs, and sometimes in the endocardium of the heart. It can also be detected in the spleen, the liver, the adrenal glands, the kidneys, the parenchyma of the salivary and lymph glands, etc.

The spirochete is neither excreted through the saliva from the salivary gland nor mixed into the saliva through the normal mucous membrane of the mouth cavity from its submucous source.

The excretion of the organism in the urine is comparatively rare.

The spirochete has never been detected in the intestinal contents of wild rats and guinea-pigs or in the bile of guinea-pigs.

It is transmitted to a healthy animal through a wound caused by the bite of an infected animal and we have reason to believe that in order to be transmitted it passes from the submucous source or from the circulating blood, through an abrasion in the mouth, such as frequently occurs in the wild rat at the time of the bite.

By keeping the infected and healthy animals in the same cage, we found no positive case among 11 guinea-pigs and only two positive cases among 29 mice. These two positive cases might have resulted from the bite of the infected mice. So it is doubtful that this spirochetosis can spread among the animals merely by their living together, and it is also doubtful that transmission can take place by means of such ectoparasites, as lice, fleas, mites, etc.

By feeding experiments, we could find only two positive cases among 14 guinea-pigs and four among 29 mice, so that it is natural to suppose that the infection in the positive cases resulted from an injured surface in the alimentary canal rather than by transmission through the normal mucous membrane.

By instillation of the infected blood into the eye, we proved only one positive case among 19 mice, and four among 14 guinea-pigs. Thus it seems hardly possible that this organism enters the animal body through the normal conjunctiva, at least, in the case of the mouse.

The Japanese monkey, guinea-pig, wild rat, white rat and mouse are susceptible to all three strains of this spirochete. The infected guinea-pig shows the rise of temperature, decrease in weight, alopecia, etc., while the inoculated wild rat, white rat, and mouse are apparently healthy, in spite of the abundant increase of the organism in their blood. The monkey inoculated with the human or wild rat strain also shows the pyrexia of relapsing type, but if the field vole strain is used, there is no fever.

The immune serum of the Japanese monkey treated with the human or wild rat strain exerts a spirocheticidal action on all three strains, while the field vole serum has only a slight action on the field vole strain, but none on the other two.

The Japanese monkey, recovered from the infection, even if it be caused by the field vole strain, does not show any further symptoms after being reinoculated with the human strain.

The spirochete under discussion does not undergo any considerable variation in virulence and immunizing power, in passing through the body of the human being, monkey, guinea-pig, wild rat, white rat, mouse, etc., but in passing through the body of the field vole, it seems to decrease its toxophore and haptophore groups quantitatively.

Our experiments prove that the human, wild rat and field vole strains all represent the same species.

We believe that Spirochaeta morsus-muris Futaki is, in all probability, similar to Spirillum minor Carter, Spirochaeta laverani Breinl, Spirochaeta muris Wenyon, etc.

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[12] Sci. Mem. Med. Off. Arm. India, 1887, 3, p. 45.

[13] Lancet, London, 1906, 2, p. 651.

[14] Mem. XXI of Liverpool School Trop. Med., 1906, 53.

[15] Jour. Hyg., 1906, 6, p. 580.

[16] Proc. Soc. Exper. Biol. and Med., 1907, 4, p. 125.

[17] Compt. rend. Soc. de biol., 1909, 66, p. 58.

[18] München. med. Wchnschr., 1908, 55, p. 1167.